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Claims

1. A macromolecular conjugate comprising a support to which is coupled at least one targeting molecule in association with an active substance, wherein said targeting molecule is biotin or an analogue thereof possessing binding activity to a biotin receptor.
2. A conjugate of claim 1, wherein the support is a polymer.
3. A conjugate of claim 1, wherein the support is a nanoparticle.
4. A conjugate of claim 1 having the general formula:
$$(B-Q)_n-P-(Q'-A)_m$$

wherein B is biotin or a derivative thereof, which is a carrier that binds to a biotin receptor

n, the molar substitution ratio of B in the conjugate, is a number from 1.0 to about 50;

P is a pharmaceutically acceptable linear, branched or dendritic polymer;

A is a pharmaceutically or diagnostic active substance;

m is a number greater than 1.0 to about 1000; and

Q and Q' are independently a covalent bond, or a spacer compound linking biotin, P and A by covalent bonds.
5. The conjugate according to claim 4, wherein at least one of Q and Q' is a spacer compound which contains a biodegradable portion.
6. The conjugate according to claim 5, wherein said biodegradable portion is selected from a disulfide bond, ester linkage, a γ -glutamyl- ϵ -lysine linkage and a diazo bond, and Gly-Phe-Leu-Gly.

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7. The conjugate according to claim 4, wherein n is from 1.0 to about 1.5 and m is from 2 to about 200, more preferably from about 10 to 100.
8. A conjugate according to claim 4, wherein P is a biodegradable polymer.
9. A conjugate according to claim 8, wherein said biodegradable polymer is selected from a biodegradable carbohydrate polymer or a polymer of amino acids.
10. A conjugate according to claim 4, wherein P is a non-biodegradable polymer.
11. A conjugate according to claim 10, wherein said non-biodegradable polymer comprises biodegradable side chains for covalent linkage to an active substance.
12. A conjugate according to claim 4, wherein said polymer is selected from poly[N-(2-hydroxypropyl)-methacrylamide], dextran or dextran derivatives, chondroitin sulfate, water soluble polyurethanes formed by covalent linkage of PEG with lysine, poly(glutamic acid), poly(hydroxypropyl glutamine), branched chain polypeptides, carboxymethyl cellulose, dendrimers and PEG-dendrimers.
13. A polymer according to claim 12, wherein said polymer is a branched chain polypeptide optionally modified to provide multiple functional groups for coupling of an active substance.
14. A conjugate according to claim 5, wherein said spacer compound Q or Q' has from 1 to 50 atoms in its backbone.
15. A conjugate according to claim 4, wherein said spacer is a diradical spacer comprising optionally substituted alkylene C₁₋₅₀ moiety optionally contained within the chain, double bonds, triple bonds, aryl groups and/or hetero atoms.

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16. A conjugate according to claim 15, wherein said spacer compound is derived from disuccinimidyl suberate (DSS), *bis*(sulfosuccinimidyl) suberate (BSS), ethylene glycol*bis*(succinimidylsuccinate) (EGS), ethylene glycol*bis*(sulfosuccinimidylsuccinate) (Sulfo-EGS), p-amino-phenylacetic acid, dithio*bis*(succinimidylpropionate) (DSP), 3,3'-dithio*bis*(sulfosuccinimidylpropionate) (DTSSP), disuccinimidyl tartarate (DST), disulfosuccinimidyl tartarate (Sulfo-DST), *bis*[2-(succinimidylloxycarbonyloxy)-ethylene]sulfone (BSOCOES), *bis*[2-(sulfosuccinimidooxycarbonyloxy)-ethylene]sulfone (Sulfo-BSOCOES), dimethyl adipimidate.2 HCl (DMA), dimethyl pimelimidate.2 HCl (DMP), or dimethyl suberimidate.2 HCl (DMS).
17. A conjugate according to claim 15, wherein said spacer compound is thiol cleavable.
18. A conjugate according to claim 17, wherein said thiol-cleavable spacer is derived from *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), iminothiolane, sulfosuccinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (Sulfo-LC-SPDP), succinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (LC-SPDP), sulfosuccinimidyl 6-[α -methyl- α -(2-pyridyldithio) toluamido]hexanoate (Sulfo-LC-SMPT), 1,4-di[3'-(2'-pyridyldithio)propionamido]butane (DPDPB), 4-succinimidylloxycarbonyl- α -methyl- α -(2-pyridyldithio)-toluene (SMPT) or dimethyl 3,3'-dithiobispropionimidate.2 HCl (DTBP).
19. A conjugate according to claim 1, wherein said active substance is a biologically active toxin or a part thereof.
20. A conjugate according to claim 19, wherein said toxin is selected from ricin, abrin, diphtheria toxin, modecin, tetanus toxin, mycotoxins, mellitin, α -amanitin, pokeweed antiviral protein and ribosome-inhibiting proteins, from wheat, barley, corn, rye, gelonin and maytansinoid.

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21. A conjugate according to claim 1, wherein said active substance is an alkylating agent selected from chlorambucil, cyclophosphamide, melphalan, cyclopropane; anthracycline antitumor antibiotics such as doxorubicin, daunomycin, adriamycin, mitomycin C, [2-(hydroxymethyl)anthraquinone]; antimetabolites such as methotrexate, dichloromethatrexate: cisplatin, carboplatin, and metallopeptides containing platinum, copper, vanadium, iron, cobalt, gold, cadmium, zinc and nickel, DON, thymidine, pentamethylmelamin, dianhydrogalactitol, 5-Methyl-THF, anguidine, maytansine, neocarzinostatin, chlorozotocin, AZQ, 2'-deoxycytosine, PALA, AD-32, *m*-AMSA and misonidazole.
22. A conjugate according to claim 1, wherein the active substance is an imaging agent.
23. A conjugate according to claim 22, wherein the imaging agent is Rhodamine, fluorescein, Texas red, Acridine Orange, Alexa Fluor (various), Allophycocyanin, 7-aminoactinomycin D, BOBO-1, BODIPY (various), Calcein, Calcium Crimson, Calcium green, Calcium Orange, 6-carboxyrhodamine 6G, Cascade blue, Cascade yellow, DAPI, DiA, DiD, DiI, DiO, DiR, ELF 97, Eosin, ER Tracker Blue-White, EthD-1, Ethidium bromide, Fluo-3, Fluo-4, FM1-43, FM4-64, Fura-2, Fura Red, Hoechst 33258, Hoechst 33342, 7-hydroxy-4-methylcoumarin, Indo-1, JC-1, JC-9, JOE dye, Lissamine rhodamine B, Lucifer Yellow CH, LysoSensor Blue DND-167, LysoSensor Green, LysoSensor Yellow/Blu, LysoTracker Green FM, Magnesium Green, Marina Blue, Mitotracker Green FM, Mitotracker Orange CMTMRos, MitoTracker Red CMXRos, Monobromobimane, NBD amines, NeruoTrace 500/525 green, Nile red, Oregon Green, Pacific Blue, POP-1, Propidium iodide, Rhodamine 110, Rhodamine Red, R-Phycoerythrin, Resorfin, RH414, Rhod-2, Rhodamine Green, Rhodamine 123, ROX dye, Sodium Green, SYTO blue (various), SYTO green (Various), SYTO orange (various), SYTOX blue, SYTOX green, SYTOX orange, Tetramethylrhodamine B, TOT-1, TOT-3, X-rhod-1, YOYO-1 or YOYO-3.
24. A conjugate according to claim 1, wherein the active substance is a radionuclide.

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25. A conjugate according to claim 22 wherein the imaging agent is a radionuclide.
26. A conjugate according to claim 22 wherein the imaging agent is linked to a polymer.
27. A conjugate according to claim 22 wherein the imaging agent is incorporated within and/or coated on a surface of a nanoparticle.
28. A conjugate according to claim 4 in which the pharmaceutically acceptable polymer has the sequence of $[(\text{NH}_2\text{-Gly})_4\text{-Lys}_2\text{-Ser}_2\text{-Lys}]_n\text{-Ala-COOH}$, where $n = 1$ to 85.
29. A conjugate according to claim 4 in which the pharmaceutically acceptable polymer has the sequence of $[(\text{NH}_2\text{-X}_o)_4\text{-Lys}_2\text{-Y}_2\text{-Lys}]_n\text{-Z}_m\text{-COOH}$, where $n = 1$ to 85; $m = 1$ to 10; $o = 1$ to 10; where X is any amino acid, where Y is any amino acid, and where Z is any amino acid.
30. A conjugate according to claim 4 in which the pharmaceutically acceptable polymer has the sequence of $[(\text{NH}_2\text{-Gly})_{16}\text{-Lys}_8\text{-Lys}_4\text{-His}_4\text{-Glu}_4\text{-Lys}_2\text{-Lys}]_n\text{-Gly}_m\text{-Cys-COOH}$, where $n = 1$ to 85; where $m = 1$ to 10.
31. A conjugate according to claim 4 in which the pharmaceutically acceptable polymer has the sequence of $[(\text{NH}_2\text{-X})_{16}\text{-Lys}_8\text{-Lys}_4\text{-Y}_4\text{-Z}_4\text{-Lys}_2\text{-Lys}]_n\text{-AA}_m\text{-Cys-COOH}$, where $n = 1$ to 85; where $m = 1$ to 10; where X, Y, Z and AA represent any amino acid independent of each other.
32. A conjugate according to claim 4 wherein P is poly[N-(2-hydroxypropyl)-methacrylamide].
33. A conjugate according to claim 1 or claim 4, wherein the biotin analogue selected from iminobiotin, Biocytin hydrazide, Biotin hydrazide, biocytin, 5-(Biotinamido)pentylamine, Sulfo-NHS(n-Hydroxysuccinimidyl)-Biotin, Sulfo-HNS-hexanyl-biotin (Sulfo-NHS-LD-Biotin), NHS-Biotin, Pentafluorophenyl-biotin,

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Pentafluorophenyl-polyethylenoxide-biotin, NHS-biotin Trifluoroacetamide, NHS-Iminobiotin trifluoroacetamide, Maleimido-polyethylenoxide biotin, Maleimido-polyethylenoxide iminobiotin, desthiobiotin, and chloracetyl-biotin.

34. A conjugate according to claim 2, wherein the biotin or biotin analogue is electrostatically or covalently linked to the polymer.
35. A conjugate according to claim 3, wherein the biotin or biotin analogue physically coats a surface of the nanoparticle.
36. A conjugate according to claim 35, wherein the biotin or biotin analogue physically coats the surface of the nanoparticle via electrostatic bonding, hydrogen bonding or hydrophobic bonding.
37. A conjugate according to claim 3, wherein the biotin or biotin analogue is attached to the nanoparticle by covalent bonding.
38. A conjugate according to claim 1, wherein the biotin analogue has cytotoxic or anti-inflammatory activity.
39. A process for synthesising a polymeric conjugate, comprising one or more of the following steps:
 - a) reacting an active substance with a polymer to form said conjugate;
 - b) chemically modifying the active substance to provide at least one functional group capable of forming a chemical linkage, and reacting the active substance and polymer to form said conjugate;
 - c) chemically modifying a target molecule, which is biotin or an analogue thereof, to provide at least one functional group capable of forming a chemical linkage and reacting the target molecule and polymer to form said conjugate;

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- d) chemically modifying the active substance and the polymer to provide functional groups capable of forming a chemical linkage, and reacting the active substance and polymer to form said conjugate;
- e) reacting the active substance with at least one cross-linking agent and reacting the active substance of polymer to form said conjugate;
- f) reacting the target molecule with at least one cross-linking agent and reacting the polymer and target molecule to form said conjugate;
- g) reacting the active substance and polymer with at least one cross-linking agent and reacting the active substance and polymer to form said conjugate;
- h) reacting the active substance directly with a polymeric support to form an intermediate containing one or more molecules of the active substance linked to the polymer, and subsequently coupling the polymer-active substance intermediate to one or more target molecules;
- i) coupling one or more target molecules to a polymeric support and subsequently reacting the carrier-polymer intermediate with one or more molecules of the active substance to give a final conjugate containing one or more molecules of the active substance.

- 40. A conjugate according to claim 1, wherein biotin or an analogue thereof is a first targeting molecule, further comprising one or more second targeting molecules, the second targeting molecules perform a helper function for biotin-binding reactions necessary for uptake and/or transport of biotin in a cell.
- 41. A conjugate according to claim 1, wherein biotin or an analogue thereof is a first targeting molecule, further comprising one or more second targeting molecules, wherein the second targeting molecules assist in release of the active substance from the conjugate in a cell.
- 42. A conjugate according to claim 1, wherein biotin or an analogue thereof is a first targeting molecule, further comprising one or more second targeting molecules,

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wherein the second targeting molecules promote a biological activity of the active substance.

43. A process for the production of a conjugate having the general formula $(B-Q)_n-P-(Q'-A)_m$ wherein B, Q, P, Q', A, n and m are as defined in claim 4, said process selected from any one or more of the following steps:
- a) reacting A with P to form an intermediate conjugate, and thereafter reacting the intermediate conjugate with biotin;
 - b) reacting biotin with P to form an intermediate conjugate and thereafter reacting the intermediate complex with A;
 - c) the process of step a) or step b) wherein one or more of biotin, P or A are modified to provide at least one functional group capable of forming a chemical linkage prior to coupling with the other reactants; and
 - d) reacting one or two of biotin, P or A with Q and/or Q' prior to coupling with the other reactants.
44. A process according to claim 43 wherein Q and/or Q' comprises an optionally substituted alkylene C₁₋₅₀ moiety optionally within the chain, double bonds, triple bonds, aryl groups, and/or hetero atoms.
45. A process according to claim 43 wherein Q' is a cleavable cross-linking agent containing a disulfide bond.
46. A process according to claim 45 wherein the cross-linking agents are selected from disuccinimidyl suberate (DSS), *bis*(sulfosuccinimidyl) suberate (BSS), ethylene glycol*bis*(succinimidylsuccinate) (EGS), ethylene glycol*bis*(sulfosuccinimidylsuccinate) (Sulfo-EGS), p-amino-phenylacetic acid, dithio*bis*(succinimidylpropionate) (DSP), 3,3'-dithio*bis*(sulfosuccinimidylpropionate) (DTSSP), disuccinimidyl tartarate (DST), disulfosuccinimidyl tartarate (Sulfo-DST), *bis*[2-(succinimidyloxycarbonyloxy)-

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ethylene)sulfone (BSOCOES), *bis*[2-(sulfosuccinimidooxycarbonyloxy)-ethylene)sulfone (Sulfo-BSOCOES), dimethyl adipimide.2 HCl (DMA), dimethyl pimelimide.2 HCl (DMP), dimethyl suberimide.2 HCl (DMS).

47. A process according to claim 43 wherein said spacer is selected from disuccinimidyl suberate (DSS), *bis*(sulfosuccinimidyl) suberate (BSS), ethylene glycol*bis*(succinimidylsuccinate) (EGS), ethylene glycol*bis*(sulfosuccinimidylsuccinate) (Sulfo-EGS), p-amino-phenylacetic acid, dithio*bis*(succinimidylpropionate) (DSP), 3,3'-dithio*bis*(sulfosuccinimidylpropionate) (DTSSP), disuccinimidyl tartarate (DST), disulfosuccinimidyl tartarate (Sulfo-DST), *bis*[2-(succinimidylloxycarbonyloxy)-ethylene)sulfone (BSOCOES), *bis*[2-(sulfosuccinimidooxycarbonyloxy)-ethylene)sulfone (Sulfo-BSOCOES), dimethyl adipimide.2 HCl (DMA), dimethyl pimelimide.2 HCl (DMP), dimethyl suberimide.2 HCl (DMS).
48. A process according to claim 43 wherein said spacer is selected from N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), iminothiolane, sulfosuccinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (Sulfo-LC-SPDP), succinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (LC-SPDP), sulfosuccinimidyl 6-[α -methyl- α -(2-pyridyldithio) toluamido]hexanoate (Sulfo-LC-SMPT), 1,4-di[3'-(2'-pyridyldithio)propionamido]butane (DPDPB), 4-succinimidylloxycarbonyl- α -methyl- α -(2-pyridyldithio)-toluene (SMPT), dimethyl 3,3'-dithiobispropionimide.2 HCl (DTBP).
49. A process according to claim 45 wherein the cross-linking agents are selected from N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), iminothiolane, sulfosuccinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (Sulfo-LC-SPDP), succinimidyl 6-[3-(2-pyridyldithio) propionamido] hexanoate (LC-SPDP), sulfosuccinimidyl 6-[α -methyl- α -(2-pyridyldithio) toluamido]hexanoate (Sulfo-LC-SMPT), 1,4-di[3'-(2'-pyridyldithio)propionamido]butane (DPDPB), 4-

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succinimidylloxycarbonyl- α -methyl- α -(2-pyridyldithio)-toluene (SMPT), dimethyl 3,3'dithiobispropionimide.2 HCl (DTBP).

50. A conjugate prepared by a process of claim 43.
51. A conjugate of claim 3, wherein the nanoparticle is prepared by solvent evaporation, complex coacervation, polymer/polymer incompatibility, gelation, interfacial polymerisation or thermal denaturation.
52. A conjugate of claim 3, wherein the nanoparticle is biodegradable.
53. A process for the production of a conjugate of claim 52, which process comprises one or more of the following steps:
 - a) reacting nanospheres with a targeting molecule to form the conjugate;
 - b) chemically modifying a targeting molecule to provide at least one functional group capable of forming a chemical linkage and reacting nanospheres and the modified targeting molecules to form the conjugate;
 - c) reacting nanospheres with at least one cross-linking agent to prepare "activated" nanoparticles which are reacted with a targeting molecule to form the conjugate;
 - d) reacting a targeting molecule with at least one cross-linking agent and reacting the nanospheres with the reacted targeting molecule to form the conjugate;
 - e) reacting nanospheres and a targeting molecule with at least one cross-linking agent to the conjugate;
 - f) reacting nanospheres with at least one cross-linking agent, reacting a targeting molecule with at least one cross-linking agent and reacting the reacted nanospheres and the reacted targeting molecule to form the conjugate; or
 - g) reacting a targeting molecule with at least one cross-linking agent to prepare an analogue which is reacted with a hydrophobic moiety to form a hydrophobic derivative of the targeting molecule, and then incubating the hydrophobic derivative

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of the targeting molecule with a nanosphere in such a manner that the nanosphere is coated hydrophobically with the targeting molecule.

54. A process of claim 53, wherein the cross-linking agent contains a disulfide bond or is cleavable by acid, base or periodate.
55. A process of claim 53, wherein the cross-linking agent is selected from the group consisting of N-(4-azidophenylthio)phthalimide, 4,4'-dithiobisphenylazide, dithiobis(succinimidylpropionate), dimethyl-3,3'-dithiobispropionimide.2HCl, 3,3'-dithiobis-(sulfosuccinimidylpropionate), ethyl-4-azidophenyl-1,3'-dithiopropionate, sulfosuccinimidyl-2-(m-azido-o-nitrobenzamido)-ethyl-1,3'-dithiobutyrimide.HCl, N-succinimidyl-(4-azidophenyl)-1,3'-dithiopropionate; sulfosuccinimidyl-2-(m-azido-o-nitrobenzamido)-ethyl-1,3'-dithiopropionate, sulfosuccinimidyl-2-(p-azidosalicylamido)-ethyl-1,3'-dithiopropionate, N-succinimidyl-3-(2-pyridylthio)propionate, sulfosuccinimidyl-(4-azidophenyldithio)propionate, 2-iminothiolane, disuccinimidyl tartrate and bis-[2-(succinimidylloxycarbonyloxy)-ethyl]-sulfone.
56. A process of claim 53, wherein the targeting molecule is cross-linked to the nanosphere or nanoparticle by reaction of the carrier with a carbodiimide and N-hydroxysuccinimide (NHS), and then reacting the NHS derivative with a suitable functional group on the nanosphere.
57. A process of claim 53, wherein the cross-linking agent contains a biodegradable bond.
58. A process of claim 57, wherein the cross-linking agent is cleaved by an esterase, glutathione, or azo-reductase.
59. A conjugate prepared by a process of claim 53.

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60. A method for the modification of a polymeric support to introduce functional groups capable of reacting either directly with an active substance or with a chemically-modified form of the active substance, wherein a resulting polymer-active substance intermediate contains one or more molecules of the active substance, said intermediate being suitable for coupling to biotin or an analogue thereof to give a conjugate capable of amplified delivery of the active substance.
61. A pharmaceutical composition which comprises a conjugate according to any one of claims 1 to 21, 24 or 28-38 together with a pharmaceutically acceptable carrier or excipient.
62. A diagnostic imaging composition comprising a conjugate according to any one of claims 22 to 27.
63. A method for the treatment or prophylaxis of disease which comprises administering to a subject a therapeutically effective amount of a conjugate according to any one of claims 1 to 21, 24 or claim 28-38 or a composition of claim 61.
64. A method of claim 63 wherein the disease is cancer.
65. A method of claim 63, wherein the disease is an inflammatory condition.
66. A method of claim 65, wherein the disease is rheumatoid arthritis.
67. A method of claim 65, wherein the disease is Crohn's disease.
68. A method of claim 65, wherein the disease is inflammatory bowel disease.
69. A method of claim 63, wherein the disease is multiple sclerosis.

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70. Use of a conjugate according to any one of claims 1 to 21, 24, or 24 to 28 in the manufacture of a medicament.
71. Use of a conjugate according to any one of claims 22 to 27 in the manufacture of a diagnostic imaging agent.
72. A method for the diagnosis of a pathological condition which comprises administering to a subject an effective amount of a conjugate according to claim 22 to 27 or a composition according to claim 62.
73. A conjugate according to claim 4, wherein the linker is biodegradable.
74. A conjugate according to claim 4, wherein the linker is a hydrazone.
75. A conjugate according to claim 4, wherein the linker contains 5-benzoyl-valeric acid.
76. A conjugate according to claim 4, wherein the linker is biodegradable and contains a valine-citrilline dipeptide.
77. A conjugate according to claim 4, wherein the linker is biodegradable and contains a phenylalanine-lysine dipeptide.
78. A conjugate of claim 1, wherein the active substance is a drug selected from platinum derivatives.
79. A conjugate according to claim 78, wherein the platinum derivative is selected from cis-Platin, CarboPlatin, oxaliplatin, multinuclear platinate species including BBR3464 and BBR3005, transdiamminedichloroplatinum (II) (Transplatin), chlorodiethylenetriamineplatinum (II), Platinum IV compounds, spiroplatin, platinum-phosphine derivatives.

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80. A conjugate of claim 1, wherein the active substance is doxorubicin or an analogue thereof, including daunorubicin, daunomycin, epirubicin, adriamycin.
81. A conjugate of claim 1, wherein the active agent is a cytotoxin selected from anti-folates including methotrexate and dichloromethatrexate.
82. A conjugate of claim 1, wherein the biotin is a hydrazidyl derivative of biotin.
83. A conjugate of claim 1, wherein the biotin is chloracetyl biotin.
84. A conjugate of claim 1, wherein the biotin is desthiobiotin.
85. A conjugate of claim 79, wherein the biotin is desthiobiotin.
86. A conjugate of claim 1, wherein the active substance is a dolastatin derivative.
87. A conjugate of claim 86, wherein the dolastatin derivative is auristatin or monomethylauristatin.
88. A conjugate of claim 4, wherein the linker is a valine-citrilline-aminobenzyl-carbamate derivative.